STUDIES ON THE ISOLATED ISLET TISSUE OF FISH.[1] II. THE EFFECT OF ELECTROLYTES AND OTHER FACTORS ON THE OXYGEN UPTAKE OF PANCREATIC ISLET SLICES OF TOADFISH, USING THE CARTESIAN DIVER MICRORESPIROMETER

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We have undertaken a detailed characterization of the metabolism of islet tissue because we believe that these studies may provide the basis for understanding the factors which control insulin synthesis and the mechanism by which alloxan and other toxic agents selectively kill the insulin-producing cells (Lazarow, 1949). In approaching this problem we have found it convenient to use the toadfish as an experimental animal. Whereas in mammals the islet tissue is distributed throughout the pancreas in a million or more individual islets of Langerhans totaling only 1% of the pancreatic mass, in the toadfish the islet cells are segregated into one or more discrete bodies which are located in the mesentery and which are called the principal islets (Diamare, 1899; Rennie, 1905). The pancreatic acinar tissue in the toadfish does not form a definite organ; rather, it is diffusely scattered throughout the mesentery, along the bile ducts, and within the liver.

In a previous study (Lazarow and Cooperstein, 1951) we have measured the activity of certain specific enzymes (cytochrome oxidase and succinic dehydrogenase) in normal toadfish islet tissue homogenates. However, in order to characterize the over-all metabolic pathways in islet tissue, it is important to measure the endogenous oxygen uptake as well as that following the addition of specific exogenous substrates. We have therefore studied the endogenous respiration of islet tissue slices and in the present paper we are reporting the effect of varying pH, tonicity, electrolyte composition, and other factors. By means of these studies we have been able to define the conditions under which maximal respiration of the islet tissue slices occurs. This should provide a base-line for subsequent work, which will include a study of (a) the effect of various substrates known to play a role in intermediary metabolism, (b) the effect of various inhibitors and, (c) the effect of hormones and other agents which influence the blood sugar level and/or insulin secretion.

Methods

Mature toadfish, *Opsanus tau,* weighing 200 to 600 gm. were used. During the summer months the animals were kept in a running sea water tank, and they were

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killed within several weeks of the time that they were caught. During the fall and winter months the toadfish were kept in a live car for varying periods of time (1–6 months) after which they were shipped inland by air express from the Marine Biological Laboratory, Woods Hole, Massachusetts. The fish were stored for periods up to one week in an aerated sea water tank (30-gallon crock). The temperature of the sea water was maintained below 20° C. by circulating cold water through the inside of submerged lead coils.

The cartesian divers used were of the cylindrical type without a bulb and they were silicone-treated prior to use. Before the fish were killed, the divers were filled with the various liquid media to be studied; they were completely filled except for a 1–2 mm. air bubble at the very bottom of the diver. The divers were then cooled to 0° C. by placing them in a cooling block. During the intervals between manipulative procedures, the divers were stored in this cold block. After the toadfish were decapitated, the islets were dissected from the mesentery and placed on a piece of Parafilm. The connective tissue capsule surrounding the islet was removed. A petri dish cover, containing a piece of wet filter paper, was placed over the tissue; this served as a moist chamber. The razor blade used for cutting the islet slices was previously cleaned with sodium hydroxide (to remove all traces of oil), thoroughly rinsed in tap water, and finally washed in distilled water. In general the islet obtained from one toadfish was used for each day's experiment; it was cut into eight pieces, each weighing approximately 0.1 to 0.2 mg. wet weight. A slice of islet tissue was then placed at the lower air-liquid meniscus in each of eight divers with the aid of a fine stainless steel needle. Most of the liquid medium was then removed from the diver using a micro pipette; however, a cylindrical segment of medium, about 1 mm. in length, was left behind to form the tissue seal containing the islet slice. With the aid of the cartesian diver filler (Lazarow, 1950), the neck seals of sodium hydroxide, oil and flotation medium were successively placed, in the stated order, above the tissue sample. The divers were then transferred to a thermostatically controlled water bath maintained at 25° C. ± 0.01° C. The pressure was measured using a Wallace Tiernan gauge (Belleville, New Jersey) which was initially suggested and used by Claff (personal communication, 1948). This gauge was calibrated in millimeters of Brodie's solution. A compensating device (Lazarow and Bloomfield, unpublished) connected to the outer chamber of the gauge was used to minimize the effect of changes in barometric pressure. An initial reading was taken after a 20-minute equilibration period. Subsequent readings were taken at 20-minute intervals during the next hour. Thermobar divers usually showed a pressure change of less than 4 mm. per hour, whereas experimental divers showed a change up to 100 mm. per hour. At the end of the experiment the divers were removed from the water bath and the sodium hydroxide, oil and flotation medium seals were removed. The tissue slice plus the tissue seal were transferred from the diver to a smaller model of the conical-tipped micro homogenizer (Lazarow and Portis, 1951), using a capillary pipette. The tissue was homogenized in 100 μl. of water and three 25-μl. aliquots were removed for protein estimation. The protein was determined by a modification of the method of Lowry, Rosenbrough, Farr and Randall (1951). Twenty-five μl. of the sample were mixed with 250 μl. of the protein reagent; the mixture was allowed to stand at 45° C. for ten minutes and, at the end of this time, 25 μl. of the diluted phenol reagent were added. The absorption was read after 15 minutes at room temperature in the Beckman spectro-

photometer at 700 mμ. The amount of protein in the sample was determined by comparing the extinction with that obtained using a standard serum albumin solution. The metabolic activity was expressed as millimicroliters (mμl.) of oxygen taken up per microgram of protein per hour.

Results

At the time this study was begun the osmotic pressure of toadfish blood (sample obtained from the gill) had been determined and found to be equivalent to 0.19 *M* NaCl (Green, personal communication). In the first group of ex-

Table I

Effect of phosphate buffer on islet tissue respiration

Series	mμl. O_2/μg. protein/hr. in saline (0.19 *M* NaCl)			mμl. O_2/μg. protein/hr. in saline-phosphate (0.033 *M* Na_2HPO_4–KH_2PO_4, pH 7.4 + 0.144 *M* NaCl)			P**
	No. determinations	Aver.	σ*	No. determinations	Aver.	σ*	
1	11	1.42	0.80	10	2.24	0.72	.012
2	17	0.78	0.42	14	1.97	0.89	<.001
3	17	1.54	0.41	17	2.20	0.70	.001
4	—	—	—	15	2.62	1.00	—
5	—	—	—	8	2.63	0.84	—
6	—	—	—	9	2.26	1.00	—
7	—	—	—	19	2.08	0.99	—
8	—	—	—	24	2.12	0.95	—
9	—	—	—	16	2.72	0.78	—
10	—	—	—	15	2.32	0.97	—
11	—	—	—	14	2.14	0.79	—
Aver.	45	1.22†	—	161	2.28†	0.37†	

* $\sqrt{\frac{\Sigma \text{ (deviations from mean)}^2}{N}}$.

** Calculated from the formula $\text{Difference} \Big/ \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$.

† Each series taken as one figure.

periments shown in Table I, the respiration of islet tissue in 0.19 *M* NaCl was compared with that observed in a saline-phosphate buffer mixture (containing 0.144 *M* saline and 0.033 *M* Na_2HPO_4–KH_2PO_4 buffer, pH 7.4). In three series of experiments the oxygen uptake of islet tissue in the saline-phosphate mixture was 43 to 153% greater than that observed in 0.19 *M* saline. The *p* values indicated that the differences were all highly significant. Table I also shows that in a group of 11 experiments in which the saline-phosphate mixture was used, there was excellent reproducibility in the average oxygen uptake for the individual series. On the other hand, in the three series of experiments in which 0.19 *M* NaCl was

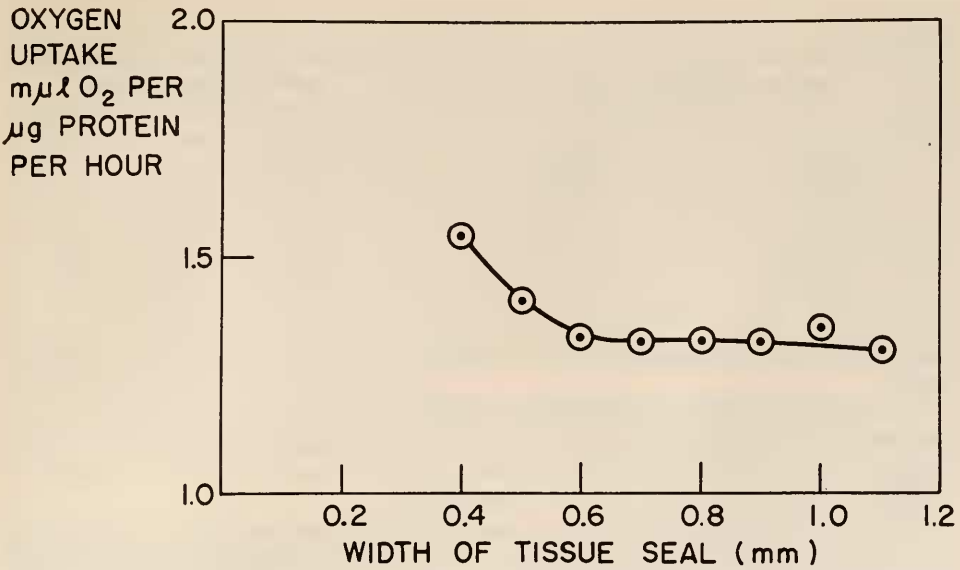

FIGURE 1. Effect of cartesian diver tissue seal width on the respiration of islet slices suspended in 0.19 *M* NaCl. All of the points on the graph in which the tissue seal width was 0.8 mm. or less represent an average of 19 or more individual determinations; the other points represent the average of 5 to 13 individual determinations.

used, the reproducibility was not as good as that observed in the saline-phosphate mixture. A possible explanation of this is shown in Figure 1. The volume of the tissue seal varied considerably from experiment to experiment and in Figure 1 the oxygen uptake of islet slices in 0.19 *M* saline was plotted against the volume of tissue seal in the diver, *i.e.*, the volume of fluid medium surrounding the tissue slice. The metabolic activity of the islet slices in 0.19 *M* saline decreased as the volume of the tissue seal increased. Moreover, this did not occur when phosphate was used; therefore, this greater metabolic variability in saline may be the result of variable dilution of the phosphate extracted from the tissue. Since phosphate addition stimulated the respiration of islet, one would expect that the oxygen uptake would be greater in the divers with small tissue seals.

The oxygen uptake of the islet tissue slices, when expressed as mμl. of oxygen per

TABLE II

Effect of different proportions of Na^+ to K^+ on islet tissue respiration. All media contained 0.033 M phosphate buffer pH 7.4 + 0.144 M NaCl or KCl. The proportions of Na^+ to K^+ were changed by varying the buffer and saline cations

Ratio Na^+/K^+ in medium	No. determinations	Aver. mμl. O_2/μg. protein/hr.	σ
100/0	16	2.77	1.26
96.5/3.5*	15	2.62	1.00

* Saline-PO_4 mixture as described in Table I.

Table III

Effect of different proportions of Na^+ to K^+ on islet tissue respiration. All media contained 0.033 M phosphate buffer pH 7.4 + 0.144 M NaCl or KCl. The proportions of Na^+ to K^+ were changed by varying the buffer and saline cations

Ratio Na^+/K^+ in medium	No. determinations	Aver. mμl. O_2/μg. protein/hr.	σ
100/0	16	2.77	1.26
80/20	16	3.24	1.46
0/100	17	3.26	1.33

μg. of tissue protein, appears to be independent of the size of islet tissue slice used.

Since the stimulation observed in the saline-phosphate buffer medium could be due either to (a) the addition of potassium ion (contained in the buffer), (b) buffering action, or (c) a specific phosphate ion effect, experiments were carried out in order to determine which factor was responsible.

The effect of varying the potassium and sodium ion concentrations of the medium is shown in Tables II and III. In the usual saline-phosphate buffer medium there are three and one half parts of potassium to ninety-six and one half parts of sodium. When an all-sodium phosphate buffer was used (prepared by mixing sodium monobasic and sodium dibasic phosphates), there was no change in metabolic activity of the islet slices. When higher potassium ion concentrations were used (Table III), the activity was about 20% higher than that observed with an all-sodium medium. However, analysis of these results showed that this difference was not statistically significant. Since the addition of phosphate buffer increased the metabolic activity of islet slices by 43 to 153%, it may be concluded that this stimulation is not due to the addition of the potassium ion contained in the buffer.

The effect of pH. Various saline-phosphate-buffer mixtures were prepared in which the pH of the phosphate buffer was varied. Table IV shows that there were no significant differences in the metabolic activity of islet slices when the pH was varied from 6.2 to 8.0; the p values were all greater than 0.3. This finding is in keeping with the studies previously reported by other investigators (Elliott and Birmingham, 1949) who suggest that the internal pH of tissue slices is maintained fairly constant over a wide range of external pH. This finding therefore suggests that the stimulatory effect observed with phosphate buffer addition (Table I) was

Table IV

Effect of pH on islet tissue slice respiration. In all cases the medium consisted of 0.144 M NaCl + 0.033 M Na_2HPO_4–KH_2PO_4 buffer

pH of medium	No. of determinations	Aver. mμl. O_2/μg. protein/hr.	σ
6.2	11	2.39	0.67
6.8	14	2.46	0.89
7.4	14	2.14	0.79
8.0	15	2.53	1.25

due to a specific phosphate ion effect rather than to the ability of the phosphate buffer to maintain the pH of the medium.

The effect of buffer type. The metabolic activity of islet slices was studied in phosphate, tris (trishydroxyaminomethane), and veronal buffers at pH 7.4 and the results are shown in Table V. The highest metabolic activity was observed with phosphate buffer. With an equimolar concentration of tris buffer, the oxygen uptake was lower than with phosphate buffer (p value $< .001$), but it was of the same order of magnitude as with 0.19 M saline (Table I). With veronal buffer the metabolic activity was 86% lower than with phosphate and much lower than with 0.19 M saline. Thus veronal buffer inhibits the metabolic activity of islet slices. Since in the presence of 0.033 M tris plus 0.033 M phosphate buffer the O_2 uptake was the same as in phosphate, the low activity in tris buffer was not due to inhibition, but rather to the absence of phosphate.

The effect of phosphate concentration. A large series of experiments were carried out in which varying concentrations of the Na_2HPO_4–KH_2PO_4 buffer (pH 7.4)

TABLE V

Effect of buffer type on islet tissue slice respiration. In all cases the medium contained 0.033 M buffer, pH 7.4; the medium containing both tris and phosphate buffer was 0.033 M with respect to each. Enough NaCl was added to each medium to maintain a tonicity equivalent to 0.19 M NaCl

Buffer in medium	Series I			Series II		
	No. determinations	Aver. mμl. O_2/μg. protein/hr.	σ	No. determinations	Aver. mμl. O_2/μg. protein/hr.	σ
Na_2HPO_4–KH_2PO_4	15	2.32	0.97	19	2.08	0.99
Tris	14	1.29	0.64	18	1.60	0.47
Veronal	6	0.33	0.13	—	—	—
Tris Na_2HPO_4–KH_2PO_4	—	—	—	19	2.02	0.52

were added and the tonicity of the medium was maintained equivalent to 0.19 M NaCl by adjusting the NaCl concentration. These results are shown in Figure 2. There was a progressive increase in the oxygen uptake as the phosphate ion concentration was increased. Maximal stimulation of metabolic activity was observed at a phosphate ion concentration of 0.066 M. The stimulation observed in the presence of 0.066 M phosphate was highly significant. A comparison of the activity in 0.066 M phosphate with that observed in the absence of phosphate, or in the presence of 0.002 M or 0.008 M phosphate, gave a p value < 0.001. The difference between 0.066 M and 0.033 M phosphate is also probably significant (p value $= 0.057$). At a phosphate ion concentration greater than 0.066 M there was no further stimulation; in fact the value at 0.136 M is actually 5% lower than at 0.066M.[2] This difference, however, is not significant ($p = 0.6$).

[2] Later studies on the effect of tonicity showed that at the high concentrations of phosphate one would have expected a large decrease in metabolic activity due to the increasing tonicity of the medium. In the presence of 0.136 M phosphate, this decrease should have been much larger than the 5% actually observed. The fact that a decrease of only 5% was observed using 0.136 M phosphate suggests that the higher concentration of phosphate is effectively stimulating the metabolic activity but that this stimulation is masked by inhibitory effects of increasing

The effect of tonicity. In order to study the effect of tonicity, the oxygen uptake of islet slices was measured in media of varying salt concentrations. However, since the phosphate buffer concentration that gives maximal stimulation (0.066 *M*) has a tonicity equivalent to 0.093 *M* NaCl, it was necessary to reduce the phosphate ion to a suboptimal value if lower tonicities were to be used. Therefore additional

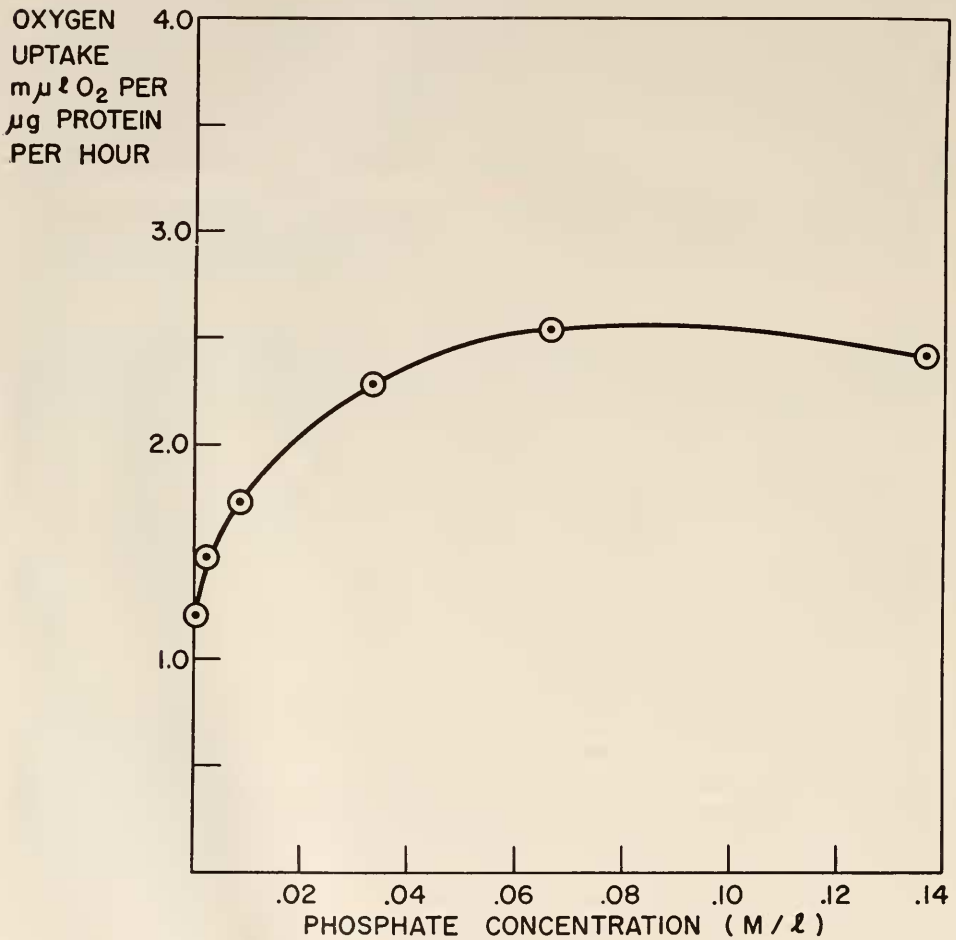

FIGURE 2. Effect of phosphate ion concentration on the respiration of toadfish islet slices. The tonicity of the medium was maintained equivalent to that of 0.19 *M* NaCl; pH = 7.4. Each of the points represents the average of 32 to 161 individual determinations.

studies were carried out with lower phosphate concentrations. Where necessary the tonicity was adjusted by adding NaCl. Table VI shows that the oxygen uptake increased with decreasing tonicity. When the tonicity of the medium was equivalent to 0.093 *M* NaCl, the metabolic activity of islet slices was 60% greater ($p = 0.006$)

tonicity. Thus the appearance of a maximum at a phosphate concentration of 0.066 *M* may be more apparent than real.

than that observed when the tonicity was equivalent to 0.19 *M* NaCl. When the tonicity was decreased further, the oxygen uptake diminished. Furthermore it should be noted that at a tonicity equivalent to 0.093 *M* NaCl, a progressive decrease in the phosphate ion concentration from 0.066 to 0.033 to 0.017 *M* gave a slight but progressive decrease in metabolic activity of the islet slices. This decrease is of the order of magnitude that would be expected from the phosphate curve (Fig. 2).

In order to explore more fully the effect of the tonicity of the medium, a series of experiments were carried out in which the phosphate ion concentration was maintained at 0.033 *M* (a slightly sub-optimal phosphate level) and in which the tonicity was varied between a sodium chloride equivalent of 0.048 *M* and 0.50 *M*. The results are shown in Figure 3. The maximum activity was observed at a tonicity equivalent to 0.075 *M* NaCl. At higher or lower tonicities the oxygen uptake was less than this optimal value. On statistical analysis the differences between the oxygen uptake at a tonicity equivalent to 0.075 *M* NaCl and those at the following

TABLE VI

Effect of varying tonicity on islet tissue slice respiration in phosphate buffer. The media contained varying concentrations of Na_2HPO_4–KH_2PO_4 buffer, pH 7.4; the proper tonicity was attained by adding the appropriate concentration of NaCl

PO_4 concentration (*M*)	Tonicity of medium (equivalent NaCl concentration) *M/l*	No. determinations	Average mμl. O_2/μg. protein/hr.	σ
0.066	0.76	8	1.84	0.93
0.066	0.380	8	2.37	0.92
0.066	0.190	8	2.68	1.18
0.066	0.093	8	4.43	0.91
0.033	0.190	8	2.62	0.84
0.033	0.093	8	4.10	1.28
0.033	0.047	8	3.16	1.05
0.017	0.093	8	3.35	0.93

tonicities were found to be significant: 0.047 *M* ($p = 0.036$), 0.147 *M* ($p = 0.036$), 0.190 *M* ($p = 0.03$). Although the differences between the oxygen uptake at 0.075 *M* and those at the other tonicity values were not significant on statistical analysis, these values nevertheless fall into a smooth curve (Fig. 3). Although a more precise localization of the maximum could be determined if a larger number of studies were carried out, there is little doubt that maximum stimulation occurs at or near a tonicity equivalent to 0.075 *M* NaCl.

Since 0.066 *M* phosphate buffer has a tonicity equivalent to 0.093 *M* NaCl, it is obvious that one cannot simultaneously achieve the conditions for both optimal phosphate and optimal tonicity. In order to achieve the most effective compromise, we have compared the respiration of islet tissue slices at pH 7.4 and at optimal phosphate sub-optimal tonicity (0.066 *M* Na_2HPO_4–KH_2PO_4 buffer, tonicity equivalent to 0.093 *M* NaCl) with that obtained at sub-optimal phosphate-optimal tonicity (0.054 *M* Na_2HPO_4–KH_2PO_4 buffer, tonicity equivalent to 0.075 *M* NaCl). The oxygen uptake in 0.054 *M* phosphate buffer was 3.54 mμl. per μg. protein per hour:

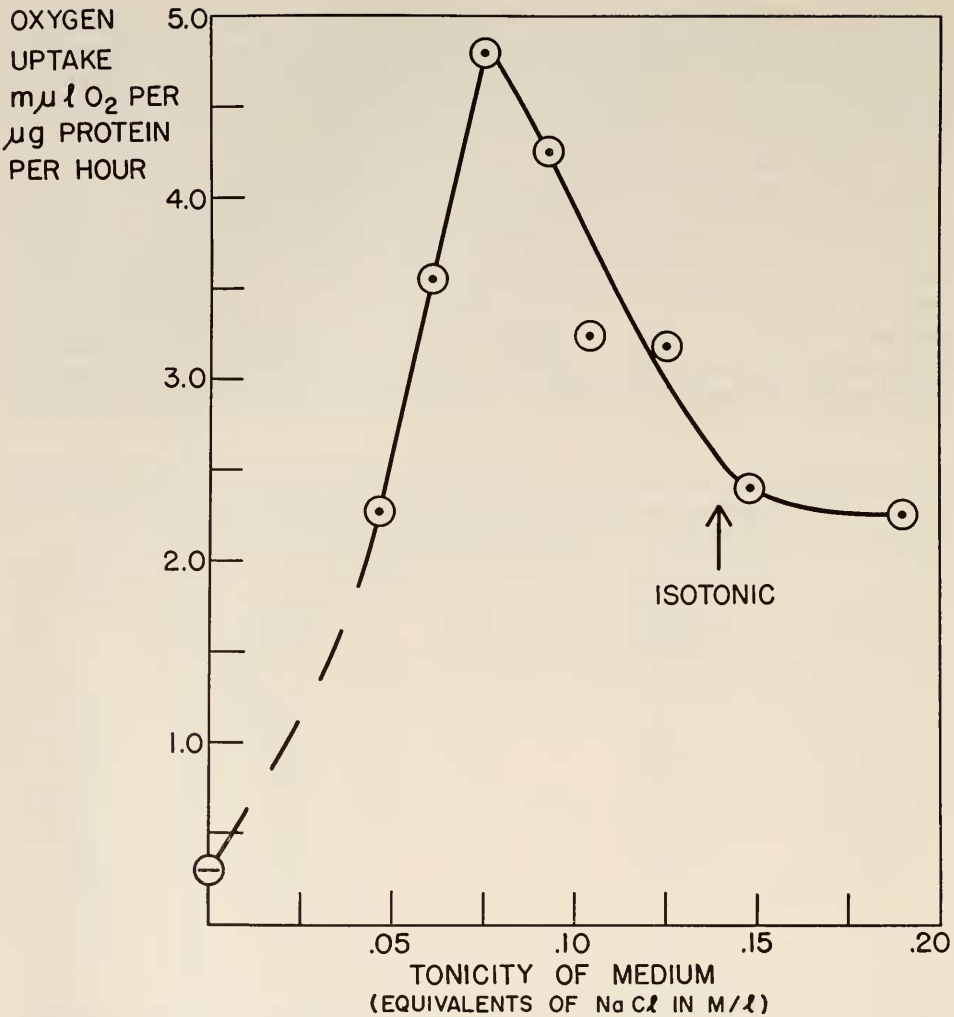

FIGURE 3. Effect of tonicity of the medium on the respiration of islet slices suspended in 0.033 *M* Na_2HPO_4—KH_2PO_4 buffer, pH 7.4. Each of the points represents the average of 8 to 10 individual determinations. The measurements at zero tonicity were carried out in distilled water and therefore this point represents the respiration in the absence of phosphate.

this was 5% greater than the activity in 0.066 *M* phosphate buffer. Although this difference is slight, it does agree with the value that would be expected on the basis of data shown in Figures 2 and 3. A decrease in the phosphate concentration from 0.006 *M* to 0.054 *M* would bring about a 3% decrease in the metabolic activity (Fig. 2), whereas a change in the tonicity of the medium from a sodium chloride equivalent of 0.093 *M* to 0.075 *M* would bring about a 13% increase in the metabolic activity. Therefore in the subsequent studies we used a 0.054 *M* Na_2HPO_4–KH_2PO_4 buffer, pH 7.4, which has a tonicity equivalent to 0.075 *M* NaCl.

The effect of calcium. Media containing a constant amount of phosphate buffer (0.054 *M*) and varying amounts of calcium chloride ranging from 0.002 *M* to 0.00005 *M* were prepared. Since the final tonicity of these solutions ranged from a sodium chloride equivalent of 0.075 *M* to 0.077 *M*, the expected changes in metabolic activity as a consequence of tonicity changes would be insignificant. The addition of calcium ion at a concentration of 0.002 *M* produced a 49% inhibition ($p < 0.001$) of oxygen uptake; at 0.001 *M* it produced a 25% inhibition ($p = 0.02$) of oxygen uptake; at concentrations of 0.0005 *M* and 0.00005 *M* it did not affect the metabolic activity.

The effect of magnesium. The effect of magnesium ion addition was tested both in the presence and absence of added calcium. Magnesium, in concentrations ranging from 0.01 *M* to 0.002 *M*, did not affect the metabolism of the islet tissue slices either in the absence or presence of calcium chloride (1×10^{-4} *M* $CaCl_2$).

The effect of trace metals. A mixture of trace metal salts, constituting the minimal trace element requirements for Neurospora growth (Beadle, 1945), was added to 0.054 *M* phosphate buffer. The final concentrations of the trace elements per liter of medium were: boron 0.01 mg., molybdenum 0.02 mg., iron 0.2 mg., manganese 0.02 mg., and zinc 2.0 mg. These metals were added as the following salts: $Na_2B_4O_7$, $(NH_4)_2MoO_4$, $FeCl_3$, $MnCl_2$, and $ZnCl_2$. In addition, two other media were prepared in which the concentrations of the trace metals were 10 times and 100 times greater, respectively, than those listed above. The addition of the above trace metals at the minimal concentration did not affect the respiration of the islet tissue slices. When the concentration of each trace metal was increased 10-fold there was a 15% inhibition ($p = 0.44$) of the oxygen uptake; when increased 100-fold, there was a 30% inhibition ($p = 0.05$).

The effect of pyrophosphate. The respiration of islet slices was studied in a medium containing 0.005 *M* pyrophosphate plus 0.054 *M* phosphate buffer. The added pyrophosphate can act as a chelating agent and thus remove trace metal ions. Its addition, however, did not affect the respiration of islet tissue slices.

The effect of serum. Samples of toadfish blood were drawn from the gill by venipuncture and the serum separated by centrifugation. The oxygen uptake of islet slices in serum was compared with that in 0.054 *M* phosphate buffer and found to be 34% lower ($p = 0.007$). On the other hand, when the serum was previously dialyzed against three liters of 0.054 *M* phosphate buffer for 18 hours at 0° C., the oxygen uptake almost equaled (95%) that in the 0.054 *M* buffer. Part of this difference may be due to a phosphate ion effect. However, since the tonicity of toadfish serum is considerably greater than the tonicity of 0.054 *M* phosphate (equivalent to 0.075 *M* NaCl), the low values obtained in serum, and the higher values obtained in dialyzed serum, can in part be due to tonicity differences. From the results obtained in Figure 3 one would expect that an increase in tonicity to that found in serum would produce a 50% decrease in the oxygen uptake, whereas a 35% decrease was actually found. These results suggest that the addition of serum protein *per se* does not materially affect the respiration of islet slices.

Discussion

It should be noted that maximal stimulation of islet respiration was observed at a tonicity equivalent to 0.075 *M* NaCl; this is considerably lower than the tonicity

of toadfish blood.[3] Similar stimulation of the oxygen uptake of brain homogenates when suspended in hypotonic media has been reported by Elliott and Libet (1942). The activity of the succinic oxidase system is likewise increased in hypotonic media and this stimulation is believed to be a direct effect on the enzyme complex (Tyler, 1954).

The stimulation of toadfish islet metabolism by phosphate is of interest. The addition of phosphate increases the oxygen uptake of brain homogenates (Elliott and Libet, 1942); it also increases the activity of certain isolated enzyme systems (Kearney, Singer and Zastrow, 1955; *cf.* Koeppe, Boyer and Stulberg, 1956). It has been suggested that it may also play a role in the control of respiration (*cf.* Lardy and Wellman, 1952). It would therefore be of interest to determine whether the oxygen uptake of slices of other toadfish tissues is similarly stimulated by phosphate addition, or if this stimulation is limited to islet tissue. Since islet tissue contains large amounts of zinc (Okamoto, 1942), and since high concentrations of the trace metal ions inhibited the oxygen uptake of the islet tissue, the phosphate stimulation could be the result of zinc chelation. It would therefore be of interest to see if pyrophosphate or other chelating agents can substitute for phosphate.

Islet tissue contains about 11%[4] protein and therefore the oxygen uptake of islet tissue slices (3.5 mμl. of oxygen consumed per μg. of protein per hour) would be equivalent to 0.39 cc. per gram of tissue (wet weight) per hour. This observed value is about equal to the reported value for brain brei and greater than that reported for liver slices. Vernberg (1954) found that the oxygen uptake of toadfish brain brei was equal to 0.41 cc./gm./hr.; the Q_{O_2} of toadfish liver slices was equal to 0.27 cc./gm./hr. The Q_{O_2} of the toadfish islet tissue is about 10 times greater than the oxygen utilization by the intact animal. Hall (1929) has reported that the oxygen consumed by the toadfish varies directly with the oxygen tension (between a partial pressure of 0 and 115 mm. of oxygen). At the atmospheric oxygen content he found that the toadfish utilized 0.038 cc. of oxygen per gram of fish per hour. Thus the toadfish islet tissue is very active metabolically compared to the fish as a whole and to the other tissues.

Summary

1. The metabolic activity of toadfish islet slices was measured in a cartesian diver microrespirometer under varying experimental conditions. The effects of pH, specific electrolytes, tonicity, trace metals, and protein addition were studied.

2. The metabolic activity was not affected by varying the pH of the medium between 6.2 and 8.0. The addition of phosphate ion stimulated the respiration. The maximum stimulation was observed when the external medium contained 0.066 *M* phosphate.

3. The respiration of islet slices was increased when the tonicity of the suspending media was reduced; optimal respiration was observed in a hypotonic me-

[3] Green and Hoffman (1953) have measured the osmotic pressure of blood samples obtained from the heart and found them to be equivalent to 0.14 *M* NaCl; the osmotic pressure of blood samples obtained from the gill were equivalent to 0.19 *M* NaCl. These authors consider the tonicity values of the heart blood samples to be the more accurate, for the gill blood samples may have been contaminated with sea water.

[4] The protein content of 5 samples of islet tissue was measured and found to contain 11.2% protein.

dium with a tonicity equivalent to 0.075 *M* NaCl; this corresponds to a phosphate buffer concentration of 0.054 *M*. This is slightly sub-optimal with respect to phosphate ion concentration. Since the metabolic activity of islet tissue slices suspended in 0.054 *M* phosphate buffer (optimal tonicity but sub-optimal phosphate) is slightly greater than in 0.066 *M* phosphate buffer (optimal phosphate but sub-optimal tonicity), subsequent studies were carried out using 0.054 *M* phosphate buffer.

4. High concentrations of calcium (0.001–0.002 *M*) inhibited the respiration of islet slices. The addition of serum protein, lower concentrations of calcium ion (0.0005 *M*), magnesium ion (0.0002–0.01 *M*), and small amounts of trace metals (boron, molybdenum, iron, manganese, zinc) did not stimulate the respiration of islet tissue slices.

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